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A preliminary field trial to compare control techniques for invasive *Berberis aquifolium* in Belgian coastal dunes

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Abstract

Non-native *Berberis aquifolium* is an invasive species in Belgian coastal dunes. With its strong clonal growth through suckers, this evergreen shrub outcompetes native species and affects dune succession. To prevent further secondary spread and mitigate its impact, there was an urgent need for knowledge on the effectiveness of control measures, both at the plant and habitat level. Here, we report on a first control experiment. Individual *B. aquifolium* clones were subjected to one of four treatments (manual uprooting, foliar herbicide application, stem cutting followed by herbicide or salt application), with regrowth being measured up to one year after treatment. We analyzed the relationship between kill rate, treatment, dune area, plant volume and number of plant stems using a generalized linear model. *Berberis aquifolium* plants proved most susceptible to foliar herbicide application (5% glyphosate solution), resulting in 88% (64%–97%) of the clones dying after treatment. The predicted kill rate decreased with an increasing number of stems under all treatments. We discuss the limitations of our experiment and the potential for actual field application of the different treatments. We present some guidelines for future control that may become further refined as experience builds up and we provide some recommendations for tackling invasive alien species in Atlantic dune ecosystems.

Keywords

control, glyphosate, invasive alien species, Mahonia, management, oregon-grape, removal, shrub

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Introduction

The Belgian coastal dunes form a dynamic and diverse ecosystem that is home to a large number of characteristic species, many of which are regionally threatened (Provoost and Bonte 2004). Embryonic dunes, shifting white dunes, moss dunes, dune grasslands and dune slacks are considered high conservation value habitats of European importance (European Commission 2013). However, the dunes are highly fragmented, making them susceptible to external influences (Zwaenepoel 2009). Invasive non-native plant species are considered one of the most important threats to their biodiversity. The amount of non-native flora within the area has increased from about 5 to 20% since the 1970's, mainly representing garden escapes (Rappé et al. 1996; Provoost et al. 2010). Non-native shrubs and trees in particular are considered problematic invaders for the open habitats of the dune ecosystem (Table 1). All of these established species were deliberately introduced for ornamental purposes and are found as escapes from gardens, garden waste dumps or public plantings (Verloove 2006). Current populations of Oregon-grape *Berberis aquifolium* Pursh. (Berberidaceae), formerly known as *Mahonia aquifolium* Nutt. (Adhikari et al. 2015), in the Belgian coastal dunes are evergreen shrubs with pinnate leaves, yellow flowers and fleshy fruits. Plants can grow up to two meters, are many-stemmed and stoloniferous. The exact origin and taxonomy of most individuals in the wild is uncertain. Many of the invasive *Berberis* populations in central Europe may have arisen from hybridized cultivars of *B. aquifolium* with *Berberis repens* Lindl. or *B. pinnata* Lag., which belong to the compound-leaved *Berberis* spp. and originate from North America (Adhikari et al. 2015). They have been subject to selection for ornamental purposes, e.g. for faster growth rate, reproductive versatility, stress tolerance, pathogen resistance and greater biomass production (Jäger and Werner 2005; Ross and Auge 2008; Ross et al. 2008). Indeed, Ross et al. (2008) showed that the majority of cultivars and invasive populations in Germany formed a gene pool different from the native species. Common garden experiments showed that plants from invasive populations in central Europe grew larger in terms of stem length, number of leaves and above-ground biomass than either of the two native species (Ross 2009). Ross (2009) therefore concludes hybridization and subsequent selection by breeders have led to an evolutionary increase of plant vigor in the introduced range. The Belgian coastal populations are extensively suckering and have leaves with numerous leaflets which according to some authors suggests introgression from *B. repens* (Verloove 2018). However, Ross et al. (2008) could not find evidence of hybridization of *B. aquifolium* and *B. repens*. In this article, we will use the name *Berberis aquifolium* awaiting further evidence on the genetic identity of invasive coastal dune populations in Belgium but in order to clearly discriminate from native *B. vulgaris* L.

Berberis aquifolium is a successful neophyte that colonizes both natural and anthropogenic habitats and is found in a wide range of habitat types (grasslands, forests, coastal dunes …) where it shows remarkable phenotypic plasticity (Ross et al. 2009). The species is shade tolerant and prefers dry to slightly moist, calcareous soils (Verloove 2006). It seems to show reduced vitality in completely sun-exposed

Table 1. Non-native tree, liana and shrub species established within the Belgian coastal dunes, in decreasing order of occurrence (% of dune areas and infected area in square meters based on field surveys in 46 nature reserves). *Populus alba/canescens* and *P. candicans* are frequently planted non-native tree species in the dunes but were not part of the survey. The Environmental Impact Assessment score for Belgium is added (ISEIA; Branquart 2007; Vanderhoeven et al. 2015). A = black list, B = watch list; 1 = isolated populations, 2 = restricted range, 3 = widespread in Belgium.

Species	Growth form	ISEIA	Surface area (m^2)	% dune areas
Rosa rugosa	Shrub	B3	56757	63
Berberis aquifolium	Shrub	A2	34035	50
Prunus serotina	Tree	A3	5461	52
Syringa vulgaris	Shrub	–	4544	30
Ribes odoratum	Shrub	–	2986	11
Symphoricarpos spp.	Shrub	–	2874	26
Robinia pseudoacacia	Tree	–	1458	4
Cotoneaster spp.	Shrub	–	1392	41
Lycium barbarum	Shrub	–	420	15
Ailanthus altissima	Tree	A2	209	9
Tamarix spp.	Shrub	–	169	9
Elaeagnus spp.	Shrub	–	108	11
Lonicera spp.	Liana	–	106	13
Prunus spp.	Tree	–	88	13
Parthenocissus spp.	Liana	B3	83	4
Ligustrum ovalifolium	Shrub	–	72	9
Ribes sanguineum	Shrub	–	58	26
Amelanchier spp.	Shrub	–	44	9
Yucca spp.	Tree-like succulent	–	25	15
Cornus spp.	Shrub	–	15	24
Baccharis halimifolia	Shrub	A1	13	11
Buddleja davidii	Shrub	B3	7	7
Euonymus japonica	Shrub	–	5	2
Pseudosasa japonica	Shrub	–	4	2
Rosa spp.	Shrub	A3	4	4
Quercus spp.	Tree	–	3	4
Sorbus spp.	Tree	–	2	4
Viburnum spp.	Shrub	–	1	2

conditions (personal observation TA). In the Netherlands, *B. aquifolium* occurs in open dunes and dune woodland and is sometimes controlled in nature reserves (personal communication J. van Valkenburg). In central and eastern Germany, *B. aquifolium* is considered an aggressive invader and one of the most important invasive shrub species in forests (Auge and Brandl 1997; K. Schneider personal communication). In Switzerland, where it is present in the Jura, Plateau and south of the Alps, it invaded forests and ruderal sites (Wittenberg 2006). In Switzerland, there is also concern about the species contributing to higher abundance of the native fruit fly *Rhagoletis meigenii* Loew (Diptera: Tephritidae), a seed predator of the native shrub *Berberis vulgaris* L. This could lead to indirect effects on its original host, its parasitoids, and other hosts of those parasitoids (Soldaat and Auge 1998). In France, Germany and Belgium, it also occurs on calcicolous grasslands, which are a high conservation value habitat

in Europe (European Commission 2013). The recent expansion of the species or its hybrids/cultivars in Western Europe is possibly linked to global warming (Walther 2002). Generally, evergreen broad-leaved species of the laurophyllous plant functional type such as *B. julianae* Schneid., *Prunus laurocerasus* L., *Lonicera nitida* Wilson and *Cotoneaster* spp. can become more competitive as a lengthened growing season (to about 300–320 days without frost) releases them from climatic constraints on their establishment potential (Walther 2000, 2002; Keil and Loos 2005).

In Belgium, *B. aquifolium* was first recorded in the wild in 1906 and naturalized in the period 1920–1950 (Verloove 2002). Its distribution only increased rapidly since the 1990s; the reasons for this increase are unknown. The highest densities are found in the northern part of the country, notably in urban environments and in the coastal dunes (Verloove 2002, 2006; Van Landuyt et al. 2012; Fig. 1). The first observation along the coast dates back to 1972 (Van Landuyt et al. 2012). In urban areas it occupies a wide range of habitats such as waste land, disused industrial and railway yards, railway tracks and old walls. In the south of Belgium, it also occurs on rocky, wooded slopes (Verloove 2006). Provoost et al. (2010, 2015) mapped the distribution of non-native shrub and tree species in nature reserves along the Belgian coast using systematic surveys (Fig. 2). *Berberis aquifolium* was the most frequently encountered non-native shrub species, and the survey also indicated that gardens and public plantings bordering natural areas represent a major source of introduction (Provoost et al. 2015; Table 1).

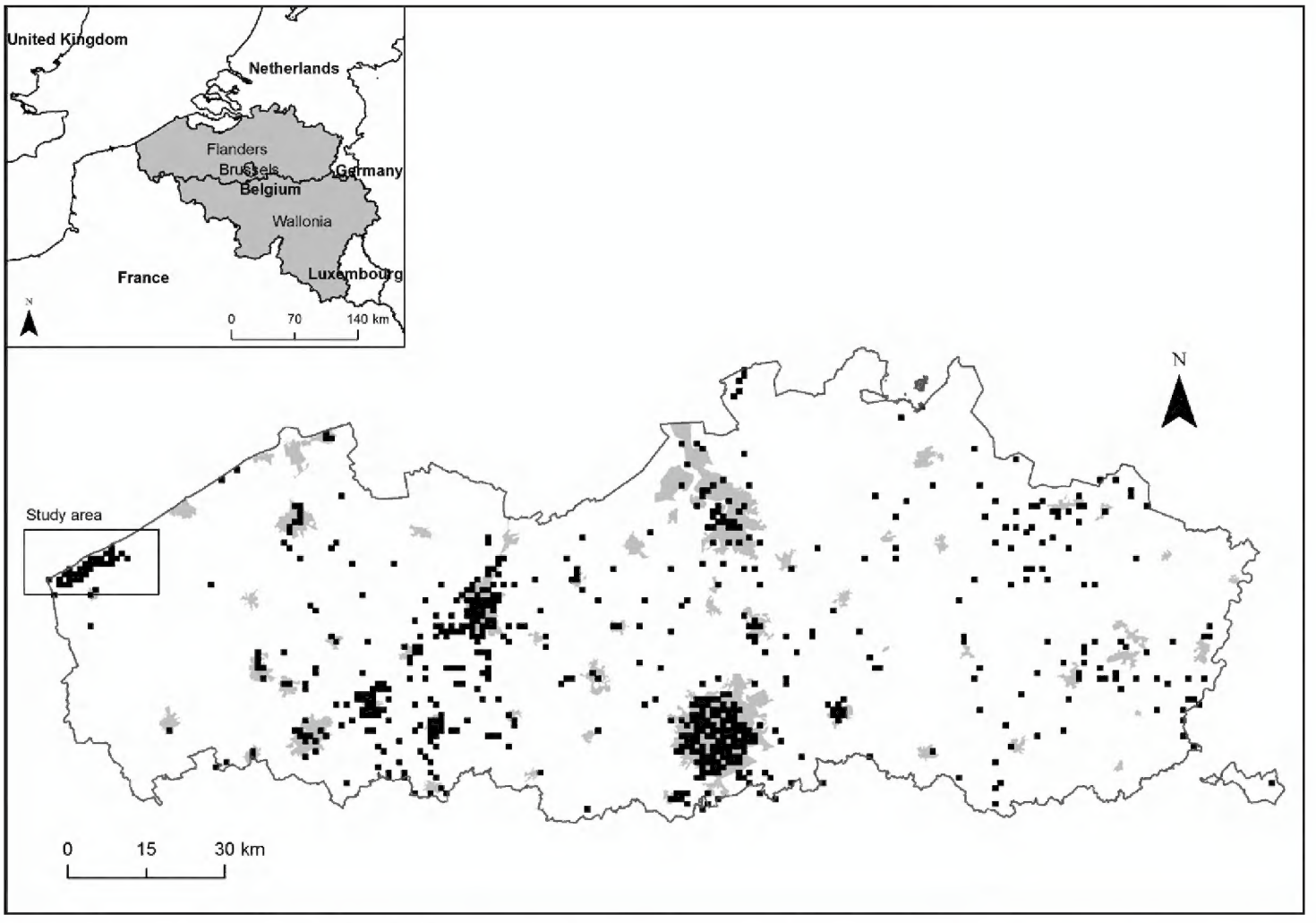

Figure 1. Distribution of *Berberis aquifolium* in Flanders at a 1 km² scale (Van Landuyt et al. 2012) with an indication of the study area. Grey color indicates urban areas. Inset: location of Belgium and its three administrative regions (Flanders, Brussels, Wallonia) in northwest Europe.

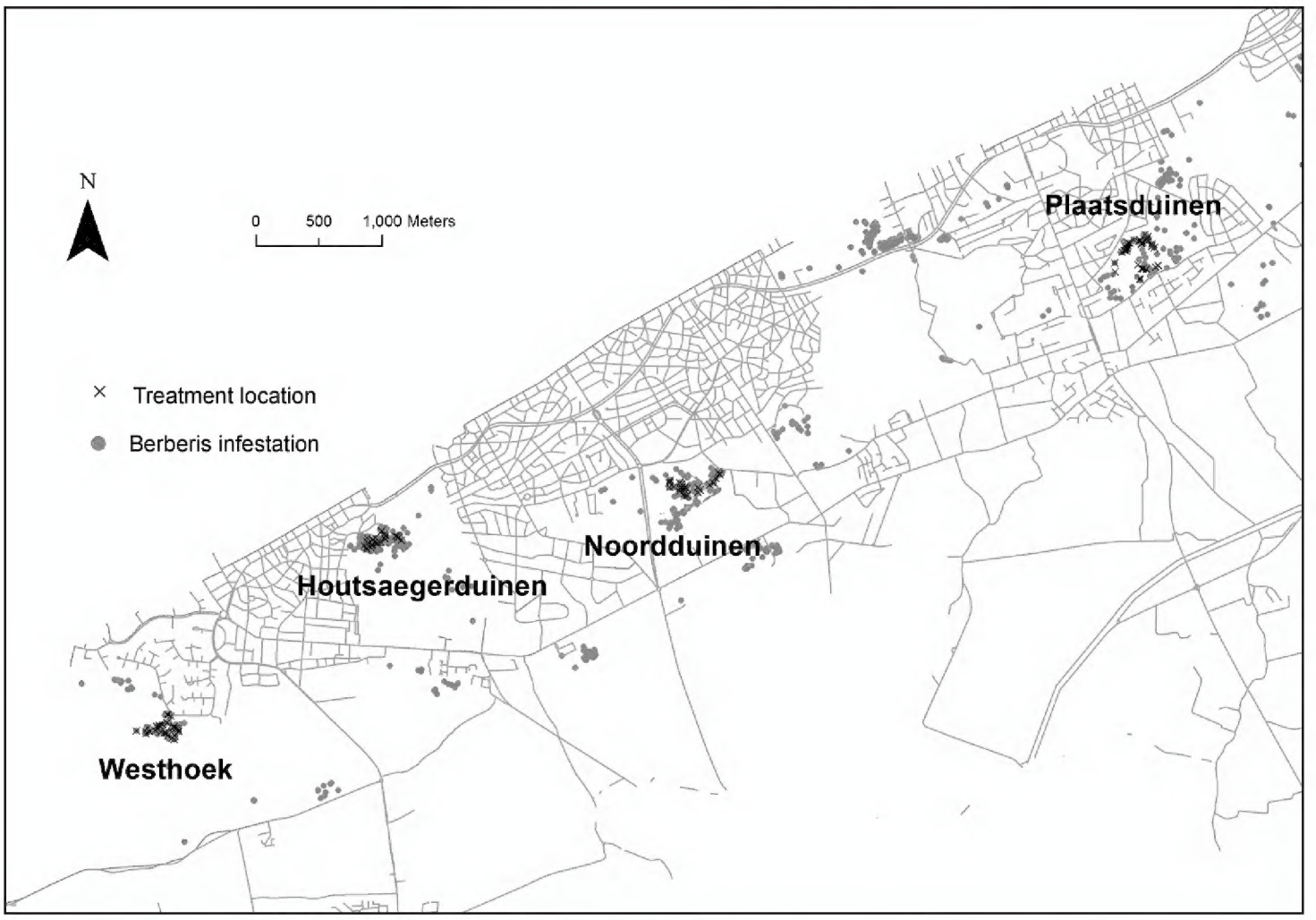

Figure 2. Distribution of *Berberis aquifolium* (2007–2015) in the study area with management trial locations within four dune sites along the Belgian coast.

It grows in a wide variety of vegetation types ranging from moss dunes and dune grasslands to scrub and woodland. Along the Belgian coast, *B. aquifolium* has invaded gray dunes (Natura 2000 habitat code 2130 sensu European Commission (2013)), dunes with sea-buckthorn *Hippophae rhamnoides* L. (habitat code 2160), *Salix repens* L. communities (2170) and dune forests (2180). *Berberis aquifolium* plants in Belgian dunes commonly host the rust fungus *Cumminsiella mirabilissima* (Peck) Nannf. but this does not seem to affect the species invasion (personal observations). The species' numerous blue berries (drupes) are easily dispersed over long distances by thrushes and other songbirds as was shown for other *Berberis* species (Silander and Klepeis 1999). Therefore, the plant can appear everywhere within the dune sites, including places which are hardly accessible to managers, such as under native *H. rhamnoides* shrub. *Berberis aquifolium* has rapid clonal growth, mostly by stem layering and below-ground stolons (Auge and Brandl 1997). Through its highly branched root system, the species attaches itself firmly in the sand. Manual pulling of mature plants can therefore be difficult and labor-intensive. As a result of its strong vegetative growth with root suckers, the species can locally appear in monospecific stands, overgrowing and displacing native species and thoroughly impacting dune succession (Verloove 2002, 2006). For instance, recent (2018) repeated vegetation mapping of the Noordduinen (Fig. 2) revealed that within 16 years, *B. aquifolium* managed to dominate and replace 2% of the indigenous scrub area (Sam Provoost, unpublished data). At the level of 50 × 50 m^2 squares, 28% of the Noordduinen is now infested with *B. aquifolium* and the species managed to dominate

and completely replace 2% of the native scrub area. As described for other scrub species in coastal dunes such as *Prunus serotina* Ehrh. in the Amsterdam Waterwork Dunes (Ehrenburg et al. 2008), a further exponential increase is expected. This perspective justifies rapid and thorough eradication. Although no specific studies exist, the impact of *B. aquifolium* in the Belgian coastal dunes is comparable to other invasive shrub and tree species for which impacts are well documented such as *P. serotina* (Ehrenburg et al. 2008), *Rosa rugosa* (Isermann 2008a, b) and *Ailanthus altissima* Swingle (Kowarik and Saumel 2007; Landenberger et al. 2007). These studies have shown negative effects on biodiversity, mainly by shading out herb-, moss- or lichen-dominated communities or by exhibiting allelopathy towards other plant species. Also, due to their higher productivity compared to native vegetation, they can alter topsoil chemical properties leading to abiotic and biotic homogenisation (Vanderhoeven et al. 2005; Dassonville et al. 2008). As a consequence, *B. aquifolium*, with *R. rugosa*, *P. serotina* and *A. altissima*, was categorized on a black list of invasive species with confirmed negative impact in Belgium following ecological impact assessment. This categorization was based on its high dispersal capacity, the colonization of high conservation value habitats by the species in Belgium, the potential for competition with native species, physical alteration of its habitat and impact on succession through accelerated colonization of open habitats by woody vegetation (Vanderhoeven et al. 2015; Table 1).

Considering the current level of infestation of the dunes (Table 1, Fig. 2), with the species being firmly established in a few publicly owned nature reserves, full eradication is considered the most appropriate management strategy. However, information on effectiveness of management measures for this species is scarce. Several projects concerning management of invasive plants in Belgian coastal dunes are planned, strengthening the need amongst conservation managers for information on effective management techniques. In practice, two main types of infestation can be considered: scattered individual shrub units (regarded as clones) on the one hand, and high density areas, almost entirely covered by *B. aquifolium* on the other. Both infestation types are present in the area and they require a different management approach. The removal of large surface areas of high density *B. aquifolium* requires landscape scale measures that often involve mechanical removal with heavy machinery. Here, we focus on a few realistically applicable manual techniques for removal of individual *B. aquifolium* clones. These clones occur scattered within the landscape and are often inaccessible to heavy machinery.

Methods

Treatment of individual plants/clones

Experimental treatment of a selection of individuals with limited clonal extension was set up in four heavily infested dune sites (Figs 1, 2; Table 2). Individual *B. aquifolium* plants selected for treatment were located with a hand-held Garmin Foretrex 401 GPS,

Table 2. Number of *Berberis aquifolium* plants treated per dune area. LEAF = glyphosate leaf treatment, DIG = manual uprooting, STUB = cut and paint glyphosate, SALT = cut and paint salt solution.

Site	Location	LEAF	DIG	STUB	SALT	Total
Westhoek	51°05'06"N, 2°33'47"E	8	9	9	7	33
Houtsaegerduinen	51°06'02"N, 2°36'10"E	7	7	5	5	24
Noordduinen	51°06'15"N, 2°37'48"E	6	7	7	5	25
Plaatsduinen	51°07'29"N, 2°41'11"E	10	10	8	8	36
Total		31	33	29	25	118

photographed and marked with a flag so as to easily relocate them in the field after treatment. Height (cm) and diameter (cm) were measured and the number of stems was counted to get an idea of the dimension of each individual or clone. Within the selected plants, clone diameter was 85 cm on average (minimum 10 cm, maximum 6 meter). In April/May 2013, plants were subjected to one of the following management treatments: (1) manual uprooting by digging with shovels (DIG), (2) leaf treatment using a spray bottle with a 5% Roundup Max (450g/l glyphosate) solution (LEAF), (3) stem treatment (cut and paint) with the same glyphosate solution (STUB) or (4) stem treatment with a saturated salt (NaCl) solution (SALT). Each ramet of a clone was treated similarly. We alternated treatments in sequence to different plants. When one plant received a given treatment, we moved to the next plant and applied the following different treatment, making sure plants were sufficiently far apart (minimally 10 meters) so as not to treat the same plant clone and making sure the most closely located plants got different treatments. The 5% glyphosate solution corresponds with the recommended concentration for cut stump treatment of *P. serotina* which is higher than the recommended concentration (1.5–2%) for leaf treatment (Agentschap voor Natuur en Bos 2013). The salt treatment is regarded as an environmentally friendly alternative because the used quantities of salt, considered on a m^2 basis, result in soil salt concentrations far below the natural values (Rozema et al. 1983). The direct effect of treatments was compared in terms of categories of stem regrowth (dead, limited regrowth, vigorous regrowth), after six months following treatment (November 2013) and after one year (May 2014). Glyphosate application was performed on rainless days with an outside air temperature below 25 °C. Since January 2015 the use of herbicides in Flanders has been banned in areas that belong to or are used for public services, areas located in drinking water protection zones, in a zone of six meters alongside surface water such as canals, waterways and ponds and on roadside verges (Decision of the Flemish Government laying down detailed rules for the reduction of pesticide use by public services of 19 December 2008). A derogation on this ban for invasive species removal is subjected to specific permits. For the purpose of this experiment we obtained permission from the competent Agency for Nature and Forest who was also the owner of the sites. In total, 127 clones were treated. Unfortunately, nine could not be retrieved, probably because flags were removed by site visitors. Nonetheless, the resulting 118 clones measured were more or less equally distributed over sites and treatments (Table 2, Fig. 2).

Data analysis

A minority of treated plants (8 out of 118) showed limited regrowth after one year. We therefore lumped limited and vigorous regrowth and considered those plants as vital after treatment. The product of plant diameter and plant height was used as a proxy for plant volume. We then investigated the relationship between kill rate (%), treatment (as a categorical variable with 4 values), dune area (as a categorical variable with 4 values), plant volume and number of stems using a generalized linear model with a binomial distribution and logit link (Quinn and Keough 2002) since we had a two-level response (dead or vital regrowth). We log transformed plant volume and number of stems to account for their skewed nature. We checked for correlations between factors in the model using Pearson's product-moment correlations (number of stems * plant volume), Pearson's Chi-squared test (dune area * treatment), Fisher's Exact Test for Count Data (regrowth * dune area and regrowth * treatment) and analysis of variance for plant volume and number of stems with dune area and treatment. We performed a multiple comparisons Tukey test to compare treatments, with a simultaneous p-value at 0.05. All analyses were performed in R version 3.1.2 (R Development Core Team 2014).

Data resources

The data underpinning the analysis reported in this paper are deposited in the Dryad Data Repository at https://doi.org/10.5061/dryad.zkh189361.

Results

Of the 118 treated plants, 45 were found dead, 8 exhibited limited regrowth and 65 were still found vital after treatment. Regrowth differed between treatments (Fisher's Exact, $p < 0.001$) but not between dune areas (Fisher's Exact, $p = 38$). Spraying *Berberis* foliage with herbicides clearly resulted in superior control with the majority of plants (26 out of 31) being killed. Salt treatment hardly affected regrowth as almost all plants (23 out of 25) remained vital after cut and paint with a salt solution. Digging (12 out of 33 killed) and stem treatment (13 out of 20 killed) showed intermediate kill rates (Fig. 3). There was an equal spread of treatments over dune areas (Pearson's Chi-squared test, Chi-square = 0.32633, df = 9, $p = 1$) and in every dune area plants with low and higher numbers of stems were treated. Regrowth was correlated with treatment (Fisher's Exact test for count data, $p < 0.001$) but not with dune area (Fisher's Exact test for count data $p = 0.38$). Number of stems and plant volume were only marginally correlated (Pearson's product-moment correlation t = 4.678, df = 125, r = 0.38 (0.22–0.52), $p < 0.001$). Treatment was not correlated with the number of stems (ANOVA: df = 3, F = 1.36, p = 0.26) nor with plant volume (ANOVA: df = 3, F = 2.15, p = 0.10). Number of stems was not correlated with dune area (ANOVA: df = 3, F= 0.06, p = 0.98). However, plant volume was not independent of dune area (ANOVA: F = 8.52,

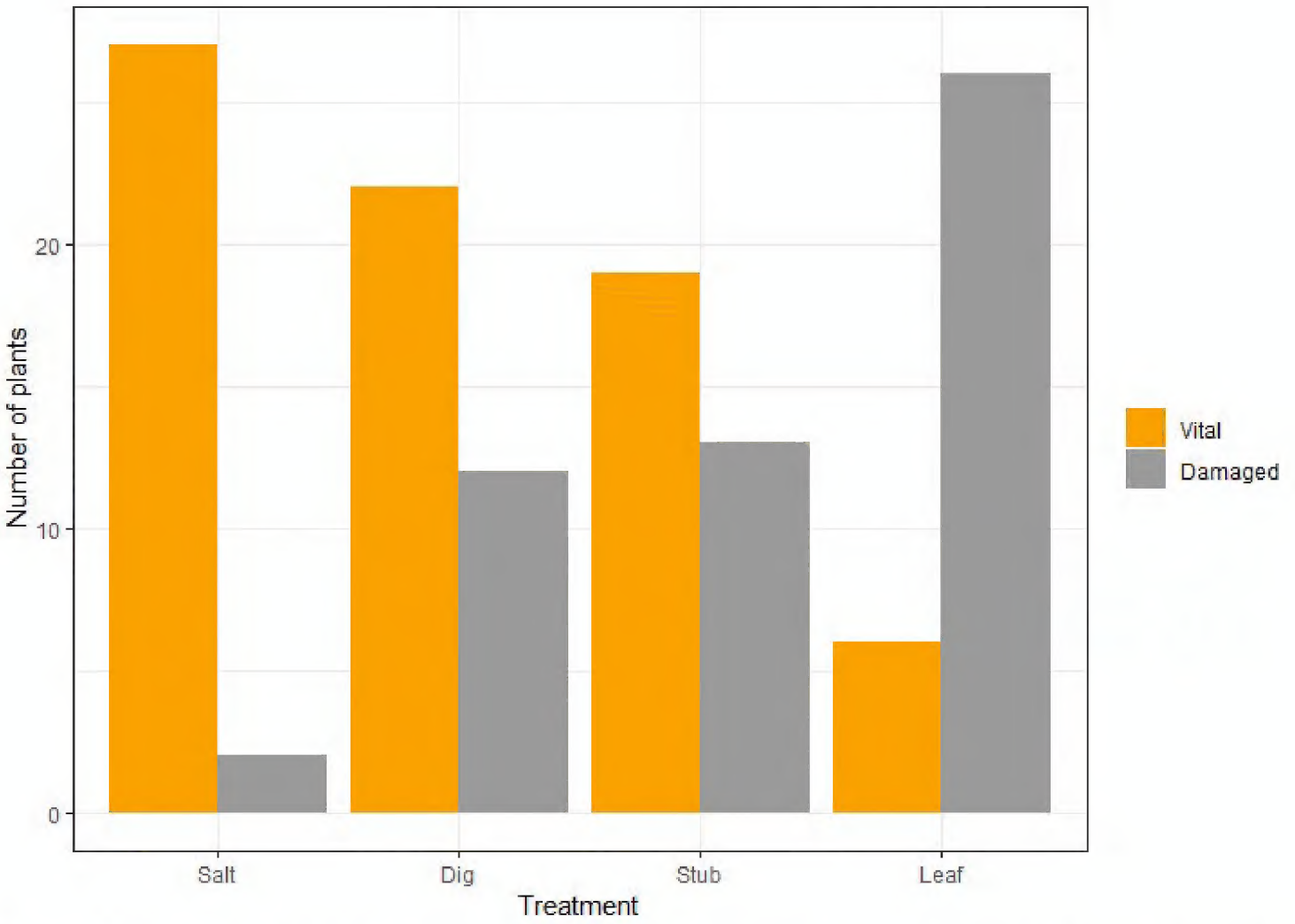

Figure 3. Number of vital and damaged *Berberis aquifolium* plants per treatment. Salt = cut and paint salt solution, Dig = manual uprooting, Stub = cut and paint glyphosate, Leaf = glyphosate leaf treatment.

$p < 0.001$), with the areas Plaatsduinen and Westhoek having bigger treated plants than Houtsaegherduinen and Noordduinen. We therefore included all factors in the generalized linear model but because of the collinearity between plant volume and area we need to be careful when interpreting effect sizes of these parameters. The predicted kill rate for *B. aquifolium* plants decreased with an increasing number of stems under all treatments. This decrease was most obvious for leaf treatment compared to other methods (Fig. 4). Leaf treatment of *B. aquifolium* resulted in superior control and was significantly different from all other treatments (Fig. 5). For leaf treatment the predicted average kill rate across all dune areas for the median amount of stems (10) and the mean log(volume) (5.2) was 88 % (95% CI: 64%–97%) (Fig. 5). The average kill rate was lower for the other treatments: 47% (19%–76%) for cut and paints and 28% (9–61%) for manual removal. Salt treatment had almost no effect with an average predicted kill rate of 4% (0.4%–26%) for salt treatment.

Discussion

Invasive species in Belgian dune ecosystems

Ornamental exotic species are increasingly causing problems for native biodiversity in Belgian coastal dunes. As the dunes are highly fragmented by urban development, the

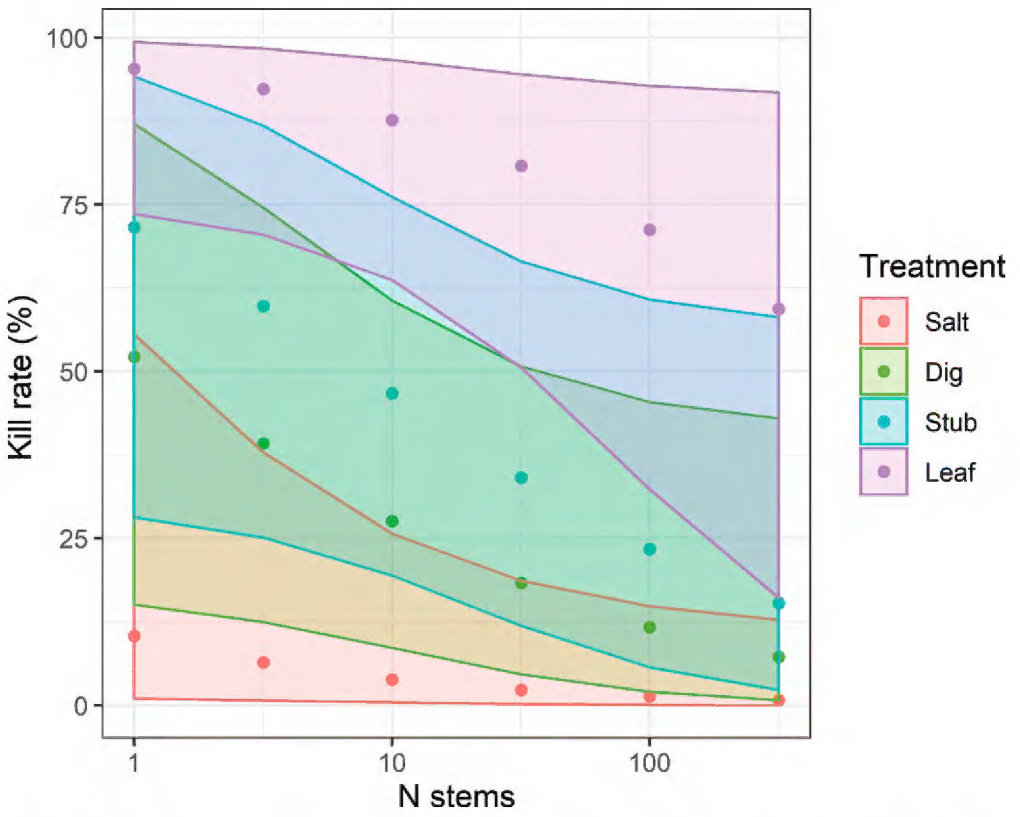

Figure 4. Modelled kill rate as a function of the number of plant stems under different treatments. Salt = cut and paint salt solution, Dig = manual uprooting, Stub = cut and paint glyphosate, Leaf = glyphosate leaf treatment.

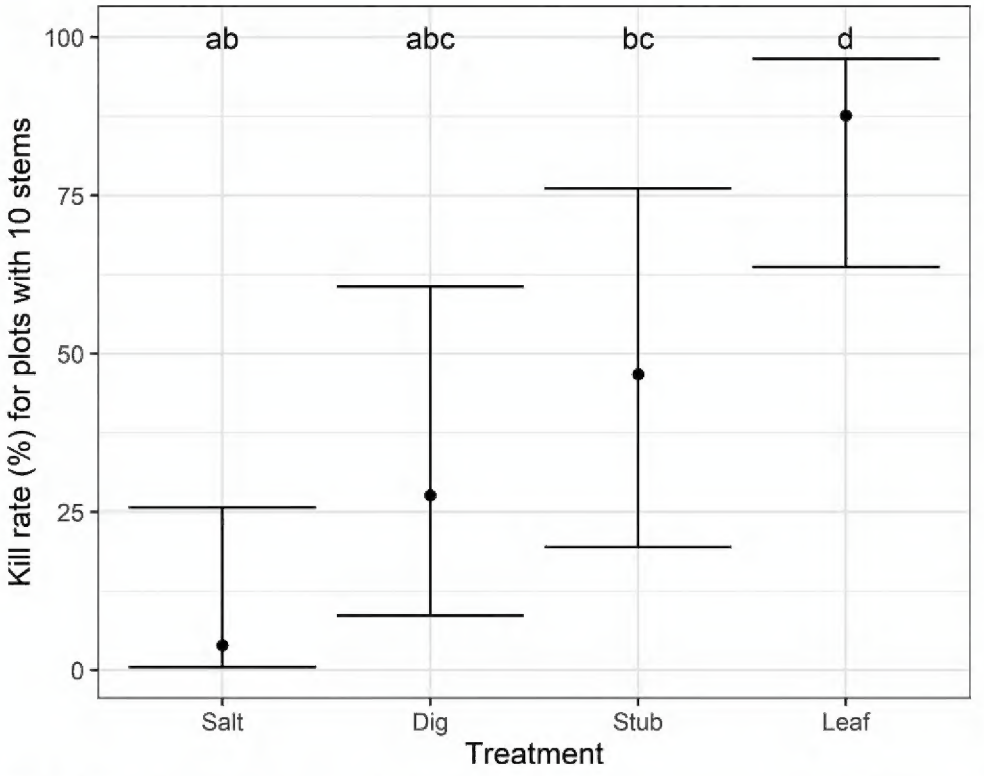

Figure 5. Modelled kill rate estimates (95% CI) for different treatments. Salt = cut and paint salt solution, Dig = manual uprooting, Stub = cut and paint glyphosate, Leaf = glyphosate leaf treatment. For ease of comparison we plotted the average predictions across all dune areas for the median amount of stems (10) and the mean log(volume) (5.2). Significant differences between treatments were tested with a post-hoc Tukey test. Treatments with the same letters above are not significantly different ($p < 0.05$).

gardens surrounding the sites are the primary source of these plant species (Verloove 2002). However, because large populations of invasives have built up within the dunes, these currently most probably act as the major secondary seed source causing further ex-

Table 3. Model outcome of the generalized linear model.

Parameter	*Est.*	SE	*P*
TreatmentSalt	0.7197	1.8788	0.70168
TreatmentDig	2.8180	1.8148	0.12048
TreatmentStub	3.5178	1.9676	0.07381
TreatmentLeaf	5.3483	1.9388	0.00581***
RegionNoordduinen	0.6100	0.6856	0.37365
RegionPlaatsduinen	0.1144	0.7596	0.88033
RegionWesthoek	1.4214	0.7336	0.05268
log(Volume)	-0.6238	0.4031	0.12171
log(N_stems)	-0.9658	0.5616	0.08546

pansion (Fig. 2). Most of the problems caused by invasions are due to, at most, 15 shrub or tree species. Differences in growth form and ecology urge for a variety of removal techniques. Important characteristics for invasiveness are a high dispersal potential and the capacity for vegetative reproduction. *Berberis aquifolium* is among the worst in these respects, given its long-distance dispersal potential through frugivorous birds and its vegetative growth potential through root suckers. It has been widely planted for its ornamental value and functionality as an evergreen hedge plant. It shows vigorous vegetative growth and has a rooting system well adapted to the coastal dune environment. Therefore, the experience with *B. aquifolium* can be useful for a wider range of other invasive species such as *B. julianae* which has recently been observed in the Belgian dunes (pers. obs. WV).

Conservation management application

Foliar application of a 5% glyphosate solution seemed by far the most effective way to remove isolated *B. aquifolium* clones, suggesting good uptake of the active compound through the stomata despite the species' glossy, leathery leaves. Manual uprooting of individuals is labor intensive and regrowth from thin root or stolon fragments is nearly inevitable. Cutting, even with glyphosate stem treatment, gives poor results and therefore seems inadequate. However, our experiments only included a single cut and we do not know the results of several years of mowing. Also, the results of the described experiment only give an impression of the aboveground regrowth after one year. Excavation of a number of individuals revealed that some roots did reshoot, even if the aboveground parts of the plant looked completely dead. Kill rates were therefore probably overestimated and retreating sites will probably be necessary in order to obtain complete removal or at least more accurate figures on the success of each treatment.

The results described here are in contrast with Stahl and Schwab (2014) who measured no effect of chemical treatment in invasive *B. aquifolium* populations of orchid-rich forest in central Germany. In this study, the herbicide used was Clinic (Nufarm) in a 33% glyphosate concentration which was applied in early July on the leaves with a paintbrush (Stahl and Schwab 2014). This higher concentration may have hindered effective uptake of the product by the leathery leaves or differences in environmental

circumstances (e.g. shade, soil conditions) might explain the contrasting results. In our study, we did not consider control plants as the emphasis was on establishing causal relationships between a set of treatments and an outcome and had hardly perceived any natural fall-out during field surveys prior to the experiment. Due to the lack of control plants, we can, however, not entirely rule out that kill rates of our glyphosate treatments would have been overestimated, although leaf-treated plants consistently turned brown within days after treatment. Furthermore, it should also be noted that the exact identity of invasive *Berberis* hybrids/cultivars in Belgian coastal dunes is unknown for the moment (see introduction). Ross et al. (2009) found no evidence for local adaptation of different populations in central Germany, but for the time being it cannot be ruled out that Belgian populations are of a different genetic constitution and therefore could react differently to management treatments.

Non-target effects of a proposed management method on the environment, economy or society are important to consider when deciding on management options for invasive species (Booy et al. 2017). This is especially true in nature reserves where the use of herbicides might impact other species of concern or may face public opposition. Since leaf application requires more glyphosate than the other control techniques, this method has more potential for non-target effects. However, visual inspections showed very little collateral damage around treated plants. This is logical as application on the leaf was performed with a hand sprayer on days with little wind and therefore was very precise. Moreover, non-target effects of the other chemical as well as mechanical treatments on non-target plant species, invertebrates and soil biodiversity cannot be ruled out and remain undocumented.

Optimizing the use of herbicides currently seems the most appropriate way to tackle *B. aquifolium*. The technique of leaf spraying is particularly useful in dunes which are inaccessible for heavy machinery, or in situations where mechanical removal using machines is inappropriate because of great conservation value or sensitivity of the local habitat. In our experiments, we followed the general advice for glyphosate application in *P. serotina* management. Herbicide treatments were performed on days without rain to prevent solution run-off from the leaves and on days with an outside air temperature below 25 °C to maximize the efficiency of the active compound glyphosate. Further experiments should be carried out, however, testing different types and concentrations of herbicide and optimal treatment timing and conditions. As *B. aquifolium* is an evergreen shrub and its flowers are conspicuous, it can easily be detected throughout the year. However, it often occurs under or in between native *Hippophae rhamnoides* where plants can be a lot harder to detect or (re)treat and where non-target effects of control are more difficult to prevent. Also, some of the dune areas are grazed by introduced cattle as a management technique. The effect of grazing on *B. aquifolium* is unknown although grazers can defoliate older plants. The choice of method should reflect on the characteristics of each site. For example, the experiments described here were performed in nature reserves and were therefore not allowed during the breeding season. Although potentially more cost effective and broadly applicable, chemical control might not be the preferred option everywhere. We acknowledge many questions

might need to be addressed before chemical control can become a viable option. For example, the use of herbicides is heavily restricted near areas used for drinking water extraction, several of which are located in the coastal dunes. Also, horizon scanning of new potentially effective compounds (e.g. triclopyr) and products (e.g. aquamaster, agridex) is often hindered by legal constraints.

As our experiment focused on individual shrubs, it offers prospects to more effectively control scattered clones which currently still represent a widespread type of infestation in Belgian dunes. However, in high density areas, almost entirely covered by *B. aquifolium*, a different management approach might be required for various reasons. The removal of large surface areas of high density *B. aquifolium* requires landscape scale measures that often involve mechanical removal with heavy machinery rather than manual removal. As a demonstration project, a heavily infested area with 100% *B. aquifolium* cover in the Noordduinen, was mechanically removed over a surface area of 350 m^2 in November 2013 using a 42 tons excavator equipped with a barred shovel aiming at sifting sand from plant material (Suppl. material 1). This was accompanied by intensive manual raking (approximately 9 man hours) which enabled the removal of most of the smaller remaining stolon fragments. Thickets and plant remains were removed using a tractor and trailer fitted with low pressure tires and using a fixed route in order to limit track formation and damage to adjacent areas. The site was revisited in May 2014 and the outcome measured in terms of *B. aquifolium* regrowth from different depths. The rooting system appeared to be relatively shallow (30–40 cm). The limited regrowth from superficially buried stolon fragments could easily be pulled out. This shows large patches of dense *B. aquifolium* can be removed mechanically. Several hundreds of square meters per day could be harvested, depending on the terrain conditions (relief) and soil moisture content. Even though the use of herbicides is strictly regulated in Flanders, and the potential for non-target effects was considered high with large-scale application, mechanical removal was preferred in this situation. Evidently, this method should fit the nature management goals of the area as it removes all vegetation and completely disturbs the soil profile. As very few native plant species can survive under the dense and evergreen *B. aquifolium* cover, botanical losses are generally limited. In our experiment, only *Rosa spinosissima* L.was of conservation concern and was also removed. Soil disturbance can also be seen as an opportunity for landscape-scale dune restoration. All over northwestern Europe, fixation and landscape senescence is seen as a threat to the specific biodiversity of coastal dunes (Arens and Geelen 2006, Provoost et al. 2011). As such, the removal of invasive plant species, and notably scrub, can be a lever for landscape rejuvenation and various management options are available (Day et al. 2003). Working in dry conditions is essential when performing this type of removal, as these facilitate the separation of soil fraction and plant material. Care should be taken to correctly dispose of the plant material which in this case involved a tractor with low tire pressure (Suppl. material 1). Manual aftercare on site and revisiting the sites during the next growing season is essential, as some regrowth of *B. aquifolium* from stolons cannot be ruled out. However, any remaining or new shoots mostly originated from superficially buried fragments and could easily be pulled out by

hand. It should be noted that only very limited regrowth was observed at the treated site six months after removal. This was still the case during field visits in summer 2015 (personal observation WVG) and is in sharp contrast with similar mechanical removals of other invasive plant species in Belgian dunes such as *Rosa rugosa* and *Ribes aureum* Lindl. which seem to have a higher potential to reshoot. The success of the mechanical scrub removal also contrasts with the manual digging treatments performed on individual clones, where large regrowth was observed. It is possible that the crane can dig up the entire root system while manual digging does not remove all of the roots.

The potential of *B. aquifolium* to become invasive in Belgian dunes was already predicted by Verloove (2002) who also advised quick removal at the time. Also, permanent monitoring of biodiversity in the Belgian coastal dunes revealed an urgent need to tackle invasive shrub invasions (Provoost et al. 2010, 2015). *Berberis aquifolium* is currently still confined to the western coastal dune areas (Fig. 2). In order to prevent its further spread to the eastern parts of the Belgian coastal dunes, removal of the current populations acting as sources of secondary spread is urgent. *Berberis aquifolium* is subject to risk communication and recommendations towards the general public as well as horticulture professionals within the framework of the Belgian Life+ project AlterIAS, in order to limit its use near habitats of high conservation value (Halford et al. 2014). This incentive should further be put into practice near coastal dunes in order to raise awareness with local horticultural stakeholders (garden centers, horticulturists, park managers etc.), public bodies and private owners to prevent the species from being planted and used in gardens and public greenery near coastal dune reserves. In parallel, the potential of promoting native alternatives such as *B. vulgaris* can be explored.

On 1 January 2015 Regulation 1143/2014 on the prevention of the introduction and spread of invasive alien species (IAS) entered into force which prohibits trade and possession of invasive species on a Union List and enforces surveillance, rapid eradication, prevention and management actions on them. However, with the exception of *B. halimifolia* L. and *A. altissima* Swingle, none of the problematic shrub and tree species mentioned in this study, nor detrimental dune invasives such as *Carpobrotus edulis* (L.) L. Bolus or *Acaena novae-zelandiae* Kirk, are on the current list of regulated species. The drafting of a list of IAS of regional concern for Atlantic dunes could be a good alternative to prevent establishment of invasive species detrimental to this unique ecosystem and to prioritize action on already established invasives. The drafting of such regional lists should be based on sound risk assessment methodologies (Gallardo et al. 2015; Roy et al. 2018) but the prioritization should also properly consider risk management options and their feasibility (Booy et al. 2017; Vanderhoeven et al. 2017).

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Supplementary material 1

Photos showing removal methods trialled on individual plant (clones) in this experiment

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Data type: multimedia

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